Synthetic Oligo *In Situ* Hybridization Probes Provide Highly Specific Target Detection Compared to Conventional Genomic-Derived Alternatives

Linda Barthel, Jacob Enk, Tina Lan, Benjamin Steil | Daicel Arbor Biosciences, Ann Arbor, MI, USA

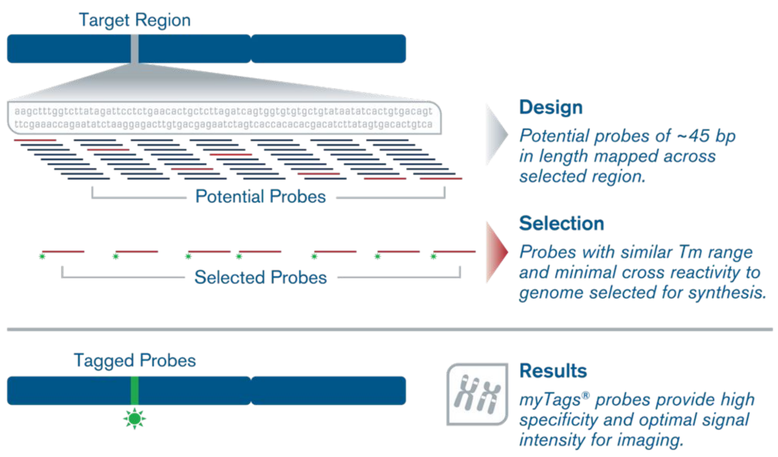
PAG30 | Booth #310

In situ hybridization (ISH) probes built from pools of synthetic single-stranded oligonucleotides are more specific and versatile than probes derived from BACs and other biological sources. The myTags® Custom ISH probe system from Daicel Arbor Biosciences utilizes a sophisticated *in silico* design process to identify and eliminate repetitive and other nonspecific elements that BAC-derived probes typically retain. Here we demonstrate the high specificity of customized myTags probe sets compared to BAC-derived alternatives, and outline design and experimental recommendations key to plant and animal genomics applications where enhanced specificity and versatility is critical. Combined with flexible synthesis and reporter labeling configurations, myTags represents an ideal and cost-effective toolset for a wide variety of genomics applications including chromosome painting and identification, haplotyping, and 3D/4D spatial genomics analysis.

Sophisticated Custom Probe System

Custom Probe Design with Specialized Algorithms

Our experts apply an advanced proprietary probe design algorithm to craft a custom solution for your targeting needs. We have decades of experience designing custom probes for a wide variety of applications



Keys to Success for Plant & Animal Applications

Versatility of myTags *in situ* Probes

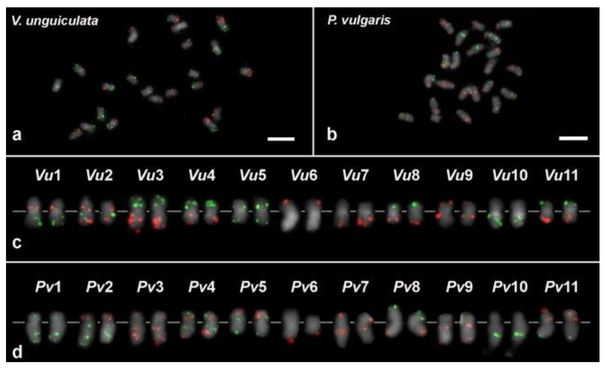
A variety of *in situ* hybridization applications in both plant and animal have benefited tremendously from synthetic oligo pools carefully designed to maximize specificity and sensitivity. myTags *in situ* probes are a highly flexible and cost-effective toolkit deploying this transformative technology. Highlighted here are some recent examples of their successful use:

Chromosome Identification

Development of chromosome identification/indexing system with oligo-FISH allows for karyotyping based on individually identified chromosomes and studies of chromosome-scale genetic adaptation and evolution.

Barcoding chromosomes in two model legume species, cowpea and common bean

Bustamante *et al.* (2021) investigated chromosome identification of V. *unguiculata* and *P. vulgaris.* Two myTags FISH probe sets (red and green) hybridized on mitotic metaphase chromosomes of *V. unguiculata* (a) and *P. vulgaris* (b). Homologous chromosomes in (a) and (b) were paired in karyograms to identify the 11 chromosome pairs of *V. unguiculata* (c) and *P. vulgaris* (d). Each chromosome shows a unique pattern of oligo-FISH red and/or green signals. Chromosomes were counterstained in DAPI (gray).



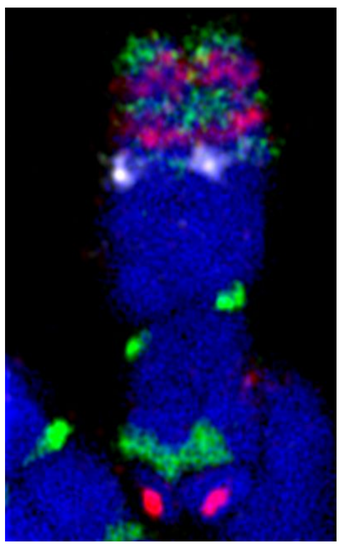
Bustamante *et al* (2021), Theor Appl Genet
Image Credit: F. Bustamante

Super-resolution Chromosome Organization

Oligo-FISH in combination with super-resolution imaging techniques allows for analyzing the subcellular localization and spatial arrangement of targeted DNA sequences at nm resolution.

FISH using myTags oligo probes reveals the helical organization of barley metaphase chromosomes.

Oligo-FISH combined with spatial super-resolution structured illumination microscopy (3D-SIM) is a useful approach for resolving helical versus non-helical arrangement of chromatin fibers in chromatids. This method was used by Randall *et al.* (2022) to confirm that the chromatids of barley metaphase chromosomes are formed by a helically wound ~400 nm chromatin fiber, termed chromonema. Additionally, by measuring the volume of oligo-FISH painted regions and based on the DNA quantity used for the probes, it was possible to calculate the chromatin compaction. These approaches determined different chromatin densities were found along the barley chromosome arm 5 HL.



Randall *et al.* (2022), Nucleus
Image Credit: V. Schubert

Synthetic vs. Genomic-Derived Oligo *in situ Probes*

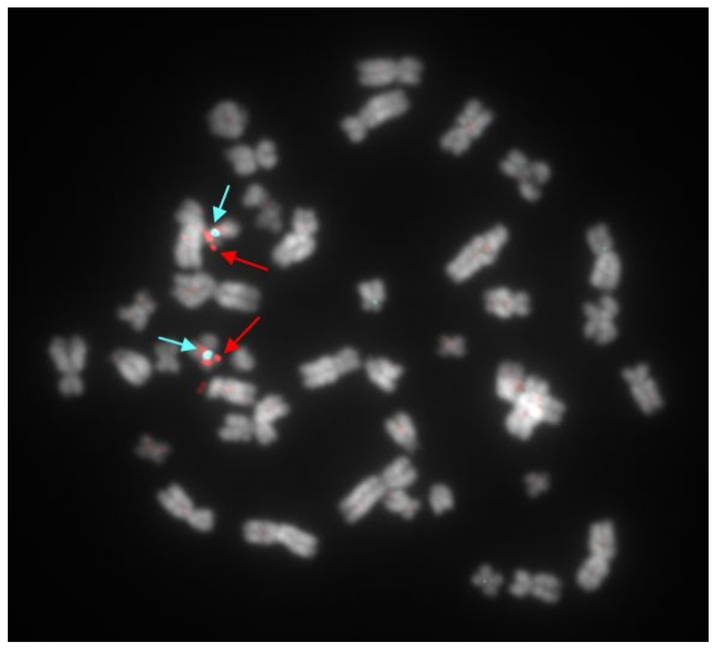
Synthetic Oligo Probes Overcome Many BAC Shortcomings

Genomic-derived probes have been a classic resource for *in situ* hybridization analysis, however synthetic probes offer many comparative advantages:

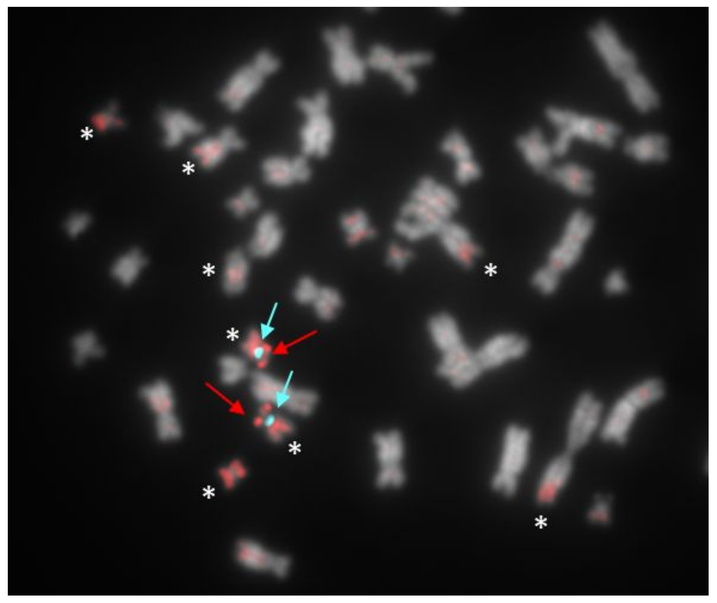
BAC-Derived Probes	myTags® Probes
• Can only be applied for DNA *in situ*	• Can be used in both DNA and RNA *in situ*
• dsDNA probes require denaturation	• ssDNA probes do not require denaturation
• Time consuming synthesis	• Rapid and reproducible synthesis
• Restricted to large target span and limited to low spatial resolution	• Customized small target span allows for high spatial resolution
• Limited target design capability	• Refined custom *in silico* design capability
• Limited specificity can result in off-target hybridization	• High-resolution design minimizes off-target errors and enables greater signal density

Synthetic Oligo Probes Demonstrate Higher Specificity

Probes hybridizing to the human TP53 loci were utilized to compare performance of BAC-derived probes and *in silico* designed synthetic myTags fluorescent probes. Human HT 1080 metaphase chromosomes were used as the target sample for standard FISH analysis. The same standard *in situ* protocol was used for all images.



myTags probes against TP53 and Chromosome 17
Standard FISH hybridization to human TP53 locus red (ATTO 550) and chromosome 17 centromere cyan (ATTO 647N). Arrows designate specific localization of hybridization signal on short arm of chromosome 17. Chromosomes are stained with Hoechst 33342 (Invitrogen), pseudo-colored gray.



BAC-derived probe against TP53 and myTags probe against Chromosome 17
Standard FISH hybridization to TP53 loci in red and chromosome 17 centromere in cyan (ATTO 647N). Arrows designate specific localization of hybridization signal on short arm of chromosome 17 and centromeric localization confirming chromosome 17 specification. Asterisks denote regions of off-target hybridization. Chromosomes are stained with Hoechst 33342 (Invitrogen), pseudo-colored gray.

References

Jiang, J., (2019). Fluorescence *in situ* hybridization in plants: Recent developments and future applications. Chromosome Res. 27, 153–165. https://doi.org/10.1007/s10577-019-09607-z

De Oliveira Bustamante, P. et al. (2021). Oligo-FISH barcode in beans: a new chromosome identification system. Theor Appl Genet 134, 3675-3686. https://doi.org/10.1007/s00122-021-03921-z

Randall R et al., (2022). Image analysis workflows to reveal the spatial organization of cell nuclei and chromosomes. Nucleus 13, 279-301. https://doi.org/10.1080/19491034.2022.2144013

web: www.arborbiosci.com
email: info@arbor.daicel.com
phone: 1-734-998-0751
twitter: @ArborBio

Custom (F)ISH Probes
arborbiosci.com/myTags
01/2023